510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k121558

B. Purpose for Submission:

New assay

C. Measurand:

C-reactive protein

D. Type of Test:

Quantitative Immunoturbidimetric

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme hsCRP POC Test Kit

Diazyme hsCRP POC control set

G. Regulatory Information:

1. Regulation section:

   C-reactive protein immunological test system 21 CFR 866.5270

2. Classification:

   Class II (assay), Class I, reserved (control materials)

3. Product code:

   DCK (assay), JJX (control materials)

4. Panel:

   Immunology (82), Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See indications for use.

2. Indication(s) for use:
   The Diazyme high sensitivity C-reactive protein (hsCRP) POC Test Kit is for the *in vitro* quantitative determination of C-reactive protein (CRP) in human venous whole blood on SMART analyzers. Measurement of CRP is of use for the detection and evaluation of inflammatory disorders and associated diseases, infection and tissue injury. For *in vitro* diagnostic use only.

   The Diazyme hsCRP POC control set is intended for use as quality controls for the Diazyme hsCRP POC Test Kit. For *in vitro* diagnostic use only.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   The Diazyme SMART analyzer (k092911)

I. Device Description:

The Diazyme hsCRP POC Test Kit system consists of the following:

Kit components
Reagent 1 40 DRS cuvette (prefilled) • 100 mM TrisCl buffer
Reagent 2 40 DRS caps (prefilled) • Suspension of anti-human CRP rabbit polyclonal antibody coated latex particles (< 0.5%).
Calibrator
1 x preprogrammed lot specific RFID card in each kit
Control Set
1 x 1.0 mL Control 1
1 x 1.0 mL Control 2

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Diazyme high sensitivity C-reactive protein (hsCRP) assay; Diazyme high sensitivity C-reactive protein (hsCRP) control kit

2. Predicate 510(k) number(s):

   k103557

3. Comparison with predicate:

Feature	Predicate k103557	Candidate device
Intended use/Indications for use	Assay: for the *in vitro* quantitative determination of C-reactive protein (CRP). Measurement of CRP is of use for the detection and evaluation of inflammatory disorders and associated diseases, infection and tissue injury. For *in vitro* diagnostic use only. Control: the control set is intended for use as quality controls for hsCRP. For *in vitro* diagnostic use only.	Same Same
Principle	Based on a latex enhanced immunoturbidimetric assay. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP which has been sensitized to latex particles, agglutination results. This agglutination is detected as an absorbance change with the magnitude of the change being proportional to the quantity of CRP in the sample.	Same
Detection	570 nm	700 nm

wavelength		

Assay range	0.2-20 mg/L	0.47 – 23.00 mg/L

Methodology	Latex enhanced immunoturbidimetric method	Same

Antibodies	Latex particles coated with goat anti-human CRP polyclonal antibodies	Same

Specimen	5μL Human serum or plasma.	20 μL Human whole blood.

Calibrator and Controls	Calibrator kit, quality control kit	RFID calibration card, quality control kit

K. Standard/Guidance Document Referenced (if applicable):

- CLSI Guideline EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods
- CLSI Guideline EP9-A2: Method Comparison and Bias Estimation Using Patient Samples
- CLSI Guideline EP7-A2: Interference Testing in Clinical Chemistry

L. Test Principle:

Diazyme's hsCRP POC Test Kit is based on a latex enhanced immunoturbidimetric assay on Diazyme's SMART analyzer. (The SMART Analyzer was cleared under k092911). When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP which has been sensitized to latex particles agglutination results. This agglutination is detected as an absorbance change (700 nm), with the magnitude of the change being proportional to the quantity of CRP in the sample. The instrument calculates the CRP concentration of patient specimen by use of a lot specific calibration curve that is stored in an RFID card provided with each hsCRP POC kit. The RFID card is inserted in the SMART analyzer and is needed for every single run.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. Precision/Reproducibility:

The precision of the Diazyme hsCRP POC Test Kit was evaluated according to Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline with the following modifications: In the study, whole blood specimens containing 0.80 mg/L, 3.25 mg/L, and 12.50 mg/L CRP were tested in 4 runs per day on three different SMART Analyzers. The results are listed below.

(1) Internal precision study performed at Diazyme Laboratories

The mean value (Mean), standard deviation, within run imprecision and total imprecision CV mg/L are calculated and summarized in the following tables:

Within Run precision

	Whole blood 1 0.80 mg/L hs-CRP	Whole blood 2 3.25 mg/L hs-CRP	Whole blood 3 12.50 mg/L hs-CRP
Total data points	40	40	40
Mean (mg/L)	0.813	3.186	12.560
SD (mg/L)	0.0782	0.0901	0.2247
%CV	9.62%	2.83%	1.79%

Within Laboratory:

	Whole blood 1 1.00 mg/L hs-CRP	Whole blood2 3.25 mg/L hs-CRP	Whole blood 3 12.50 mg/L hs-CRP
Total data points	40	40	40
Mean (mg/L)	0.813	3.186	12.560
SD (mg/L)	0.0664	0.0984	0.2667
%CV	8.17%	3.09%	2.12%

2) External precision study performed at POL sites

Precision was evaluated at three (3) physician office laboratories (POL) by intended users such as nurses and office assistants. Six (6) whole blood samples containing C Reactive Protein levels ranging from low to high were tested. At each site, 2 whole blood samples were tested. Each sample was run 4 times per day for 5 days using three SMART Analyzers.

Within run precision:

	Site 1		Site 2		Site 3	
	Whole blood 1	Whole blood 2	Whole blood 3	Whole blood 4	Whole blood 5	Whole blood 6
No. of	20	20	20	20	20	20

Points						
Mean (mg/L)	0.798	4.796	0.758	17.796	7.780	18.725
SD (mg/L)	0.0372	0.3369	0.0684	0.7447	0.4225	0.9409
CV	4.67%	7.02%	9.04%	4.18%	5.43%	5.02%

Within-laboratory precision:

	Site 1		Site 2		Site 3	
	Whole blood 1	Whole blood 2	Whole blood 3	Whole blood 4	Whole blood 5	Whole blood 6
No. of Points	20	20	20	20	20	20
Mean (mg/L)	0.798	4.796	0.758	17.796	7.780	18.752
SD (mg/L)	0.0684	0.3674	0.0616	0.7153	0.4082	0.8951
CV	8.58%	7.37%	8.13%	4.02%	5.24%	4.78%

b. Linearity/assay reportable range:

A set of eleven levels of linearity materials were prepared by diluting a whole blood sample containing 28.0 mg/L of C Reactive Protein (CRP) with saline according to Clinical and Laboratory Standards Institute EP6-A and were tested with the Diazyme C Reactive Protein POC Test in triplicate on the SMART Analyzer. Testing included samples with concentrations near the LoQ. Conclusion: Linearity data and the LOQ data support Analytical Measuring Range (AMR) of 0.47 mg/L to 23.00 mg/L.

The regression equation yielded slope of 0.996; intercept of -0.0065 and R^2 of 0.9999.

The package insert recommends diluting samples with CRP > 23.0 mg/L 1:1 with saline. To demonstrate validity, 3 whole blood samples (spiked) containing CRP near the upper detection limit of 26.0 mg/L CRP were diluted 1:1 with saline and tested on the SMART analyzer. Recoveries based on dilutions were all within 95% of the expected concentrations.

No high does hook effect was observed for samples with concentrations up to 200 ng/mL.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Calibrator

The instrument calculates the CRP concentration of patient specimen by use of a lot specific calibration curve that is stored in an RFID card provided with each hsCRP POC kit. The RFID card is inserted in the SMART analyzer and is needed for every single run.

The hsCRP POC assay calibration is traceable to Diazyme CRP calibrators (k103557) which are in turn traceable to IFCC International Reference Preparation for C Reactive Protein in the human serum reference material ERM®-DA472/IFCC.

Control material
Control material target values are assigned based on the mean of replicate values on the SMART analyzer. The range is assigned as the mean +/- 15%. Stability protocols and criteria were included within the 510(k).

Stability testing was provided to support the claim of 1 month at 2-8 degrees C for opened vials, and 12 month shelf life. Real-time stability studies are ongoing.

d. Detection limit:

LOB and LOD were calculated based on CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation using whole blood samples (except for true blanks which were phosphate buffered saline. Based on this analysis:

LoB was estimated as 0.055 mg/L. LoD was estimated as 0.15 mg/L.

To calculate the LoQ of the Diazyme hsCRP POC Test, five patient whole blood samples were diluted to concentrations of 0.1, 0.2, 0.3, 0.5, and 1.0 mg/L. The diluted samples were tested with the Diazyme hsCRP POC Test on SMART analyzers for 20 replicates over 5 days. At the claimed LoQ of 0.47 (CV observed in this evaluation was 17% and observed bias was 8.4%.

The sponsor's claimed measuring range is 0.47 – 23 mg/L.

e. Analytical specificity:

Common endogenous substance interference
Potential interferents were evaluated using whole blood samples containing

"low" (near 1 mg/dL CRP) and "high" (4-5 mg/dL CRP) C Reactive Protein spiked with various concentrations of substances according to Clinical and Laboratory Standards Institute EP7-A "Interference Testing in Clinical Chemistry": dose-response guidelines. Each sample spiked with interference substances was tested in triplicates and compared to control samples without potential interferents.

The interference substances examined and their concentrations tested are listed in the following table:

Compound tested	Maximum interferent concentration tested	Maximum interferent concentration tested/ or at which no significant* interference was observed
Ascorbic acid	176 mg/dL	176 mg/dL
Bilirubin	40 mg/dL	40mg/dL
Conjugated Bilirubin	40 mg/dL	30mg/dL.
Triglycerides	1000 mg/dL	1000 mg/dL
Hemoglobin	20 g/dL	20 g/dL
Rheumatoid Factor	375 IU/mL	250 IU/mL.

Additional potential interferents were evaluated using whole blood samples containing concentrations of CRP below1 mg/dL. Each sample spiked with interference substances was tested in triplicates and compared to control samples without potential interferents. Results are tabulated below:

Compound tested	Maximum interferent concentration tested	Maximum interferent concentration tested/ or at which no significant* interference was observed
Oxaloacetate	300 uM	200 uM
Glutathione	300 uM	200 uM
Isoniazid	300 uM	200 uM.
L-Dopa	300 uM	200 uM

* The Sponsors defines significant interference as > 10% change in recovery..

f. Assay cut-off:

Not applicable – this is a quantitative assay.

2. Comparison studies:

a. Method comparison with predicate device:

Internal testing:
A total of forty (after excluding one sample that was out of the assay detection range) EDTA whole blood specimens were tested with Diazyme hsCRP POC Test on SMART analyzer. The correspondent plasma samples were tested with Diazyme hsCRP Assay on Hitachi 917 analyzer (predicate k103557). The regression results are summarized in the following table:

	Whole blood application
n	40
Slope (w/ 95% Confidence Interval)	0.9871 (0.8122-1.0325)
Intercept (w/ 95% Confidence Interval)	-0.4004 (-0.2810 to 0.0540)
Correlation coefficient	0.9511
Range of values	0.47-22.50

POL site testing:

One hundred and twenty (120) paired human whole blood-serum samples (a tube of venous whole blood and a tube of serum from the same individual) were tested for comparison. At each site of the three sites, 40 whole blood samples were tested using SMART analyzers. The corresponding one hundred and twenty (120) plasma specimens were tested on Hitachi 917 with predicate device (K103557) at Diazyme Laboratories. One hundred and sixteen were used after excluding four samples that were out of the assay detection range. The regression results are summarized in the following table:

	Site 1	Site 2	Site 3	3POL Site Combined
n	38	39	39	116
Slope (w/ 95% Confidence	0.9195 (0.8996 - 0.9927)	1.0125 (0.9508 - 1.0484)	1.0073 (0.9017 - 1.0369)	0.9712 (0.9420 - 1.0056)

Interval)				
Intercept (w/ 95% Confidence Interval)	0.0531 (-0.0395 - 0.1689)	-0.0839 (-0.1744 - 0.1349)	-0.2795 (-0.3237 - 0.0040)	-0.0870 (-0.0463 - 0.0686)
Correlation coefficient	0.9889	0.9811	0.9858	0.9836
Range of values	0.60-19.55	0.47-14.00	0.51-22.79	0.47-22.79

b *Matrix comparison:*

Not applicable – the assay is for use in whole blood EDTA samples.

3. Clinical studies:

   a. *Clinical Sensitivity:* Not applicable; Clinical sensitivity and specificity is not typically provided in 510(k)s for this type of assay.

   b. *Clinical specificity:* See a, above.

   c. *Other clinical supportive data (when a. and b. are not applicable):*

4. Clinical cut-off**:**

   See "Expected values" Section, below.

5. Expected values/Reference range:

   To verify the transferability of the reference interval from the predicate device, whole blood samples from 150 apparently healthy individuals were tested using the Diazyme hsCRP POC Test according to CLSI C28-A3 guideline.

   The expected normal range is < 5.0 mg/L in 95% of the population tested.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.